THE VALUE OF MANGANESE IN BUILDING HEMOGLOBIN IN RATS MADE ANEMIC ON A MILK DIET

by

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INTRODUCTION

The problem of the formation and the destruction of hemoglobin in the animal body has occupied the attention of research workers for many years. Much of this experimental work has centered about the question of whether inorganic iron would cause the building of hemoglobin or whether organic iron was needed for the building of hemoglobin.

HISTORICAL

The experimental work of Hart and co-workers (9) seemed to prove that pure inorganic iron alone would not cause the building of hemoglobin when fed to young rats made anemic on a whole milk diet. They proved that inorganic iron only when accompanied with small amounts of copper salts would cause the rapid building of hemoglobin (1). Since the announcement of their results many other workers have been studying the effect of different elements on the building of hemoglobin. These investigators are unanimous in the conclusion that a mixture of inorganic iron and copper will cause rapid building of hemoglobin in experimental animals made anemic on an exclusive milk diet (5), (3), (9), (2).

A question on which worker's are not in agreement is

whether copper is the only element that will build hemoglobin when fed with iron to rats made anemic on a milk diet. Also their opinions on the value of iron alone in such cases are not the same. Dr. Mitchell of Battle Creek Sanitarium (6) and Dr. Meyers of Cleveland (2) have found that the feeding of iron alone with milk to anemic rats would cause slow building of hemoglobin. The Wisconsin workers and also Titus of this station, found that iron alone did not cause hemoglobin building (8) (9). The Wisconsin workers insist that the presence of small amounts of copper are needed with the iron to build hemoglobin and that copper is the only element that if fed with iron will cause building of hemoglobin (1), (7), (9). Waddell and co-workers of the same school tried twelve different elements with iron including manganese, arsenic and zinc, and only copper proved effective (1). The other elements gave only temporary and minimal results. R. W. Titus and co-workers of this school found that manganese combined with iron caused only a slightly less rapid building of hemoglobin than copper (8). Meyers (2) and Mitchell (6) also reported manganese as giving positive results when fed rats made anemic on an exclusive diet of milk (6), (2).

STATEMENT OF PROBLEM

In order to find the cause of the difference in the results obtained here and at Wisconsin University samples of the salts used here were sent to Dr. Hart and he in turn sent to this school samples of the salts he used. The results here again showed a marked beneficial action by the manganese salts, while he obtained negative results with the same substances. In order to check these experiments further it was agreed that Dr. Hart should send samples of his copper, manganese and iron salts to one of his former pupils, a Dr. Krauss at Wooster, Ohio. Dr. Hughes was also to send samples of the solutions which he had been using. In this way an independent worker would test the solutions on the same quality of milk and rats. Then a difference in results could not be explained as due to milk from different herds, nor to a difference in the compounds, nor to a difference in technique. In order to eliminate any of these possibilities it was agreed that the workers at both stations were to prepare solutions of copper sulphate, manganese chloride and iron chloride and send portions to Dr. Krauss at the Ohio Experiment Station at Wooster. He was to run two groups of his rats under identical conditions as far as possible, using the salts from Dr. Hart on one group of rats and the solutions from Dr. Hughes on another lot of

similar rate. The object of this experiment is to test out a portion of the solutions sent to Dr. Krauss in order to see what effect they would have on hemoglobin building when fed to rats made anemic with a milk diet.

EXPERIMENTAL WORK

Young albino rats were used in this experiment. It has been proven by Hart and Steenbock of Wisconsin University that young rats can store copper and iron of their food for future use. (1). Titus and co-worker of this station and also McHargue of Kentucky University, found that manganese in the food of young animals could be stored by them (10), (3). Because of these facts it is important in making the young rats anemic that they do not previously receive food containing more than traces of these elements. Since they begin to taste their mother's feed after about twelve days of age, their mother's diet which had contained these elements was then changed to whole wheat and milk. When the young were a month old they were weined and placed on a diet of whole milk. After about three weeks the young began to show signs of being anemic. Hemoglobin determinations were then made on their blood each week and as soon as any rat's hemoglobin registered below six grams per hundred cubic centimeters that rat was placed in a group to receive the min-

eral supplement to its milk diet.

All the milk used in this experiment was obtained from the college dairy herd directly from the cooler. All the buckets and the cooler used in the dairy were well tinned so there was no chance of contamination. The milk was obtained in glass bottles and fed in porcelain mortars so no metals could enter the milk. The iron, copper and manganese solutions were prepared according to the following directions: All water used in their preparation was redistilled from glass. Even the pipettes and dishes used in making the solutions were rinsed with this kind of distilled water. The iron chloride was prepared from 15.02 gms. of the best grade of Mallinckrodt's iron wire (for standardizing). It was dissolved in a slight excess of Baker's C. P. concentrated HCl (about 25 cc.) plus some distilled water. This solution was then diluted to about 200 cc. and heated until hot and then washed H_2S gas under pressure was passed into it for 24 hours. The excess H_2S gas was boiled out of the liquid and the very slight precipitate which might be CuS was filtered out. The filtrated with washings was diluted to 1000 cc. In making the stock feed solutions 16.6 cc. of this solution were added to each 500 cc. of solution made, so each 1 cc. would contain .5 mg. of

iron. In preparing the manganese solution 6.2751 gms. of $MnCO_3$ was weighed and dissolved in a slight excess of Baker's C. P. conc. HCL plus water and treated in the same manner as the iron solution to remove any copper that might be present. The excess H_2S gas was also removed by boiling. When making 500 cc. of a feed solution 10 cc. of this solution was used so each cubic centimeter would contain .1 mg. of manganese. The copper solution was 5.8918 gms. of Baker's analyzed C. P. $CuSO_4$, $.5H_2O$ dissolved in 500 cc. of water. Since each cubic centimeter would contain .003 gms. of copper, only 8.3 cc. were used in making each 500 cc. of feed solution so each cubic centimeter would contain .05 mg. of copper.

The rats were kept in individual cages of galvanized iron wire with screened bottoms. The minerals to be given each group were fed as one cubic centimeter of stock solution with a small amount of milk for their evening meal. In the morning when this had all been consumed enough milk was given them to last the rest of the day. Each evening feed dishes were emptied, cleaned and sterilized. Then they were rinsed with distilled water before placing in them the fresh milk and the mineral supplement. The mineral supplements were made by the needed amounts of the above stock solutions being diluted to 500 cc. so that in feeding them

only 1 cc. needed to be placed in the feed dishes.

Four lots of rats were started. Lot one received .1mg. of Mn plus .5 mg. of Fe, lot two had only .5 mg. of iron, the third group had .05 mg. of copper with .5 mg. of iron and the fourth group had the same amount of copper, iron and manganese. The rats were weighed and their hemoglobin determined each week. They were bled near the end of the tail by the usual method as given by Titus and co-workers and the hemoglobin was read on the Fleischel Miescher hemoglobinometer by Mr. Loy (8). The results for those attaining normal hemoglobin readings are given in the follpwing Table I:

TABLE I.

Group I Lot 1. Manganese(.1mg) and Iron(.5mg)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. gained per 100 cc.
1	72	4.89	14.47	98	9.58
2	104	5.50	16.00	70	10.50
3	118	5.70	15.30	50	9.60
4	93	5.50	7.35	91	died
5	102	5.90	7.08	30	died
6	75	3.87	13.47	84	9.60
7	79	6.54	9.18	7	discontinued
8	114	5.50	13.60	35	8.10
9	87	5.70	12.24	7	discontinued
10	75	5.30	10.81	64	died
11	100	6.93	12.24	105	5.31
12	118	6.93	15.09	51	8.16
13	106	5.50	14.07	51	8.57
14	106	4.69	16.92	51	12.23
15	132	4.08	3.87	7	died
16	107	4.69	6.95	14	died
17	104	4.89	11.00	37	died

TABLE I. (continued)

Group I Lot 1 Manganese(.1mg) and Iron(.5mg)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemglobin gms. gained per 100 cc.
18	173	4.89	4.69	7	died
19	114	5.70	12.84	51	7.14
20	111	3.27	3.30	7	died
21	101.5	5.10	15.70	45	10.60
22	68	5.10	9.18	24	died
23	102	3.60	5.10	16	died
24	102	4.50	4.50	8	died
25	129	4.50	13.86	39	9.36
26	175	5.70	13.47	39	7.77
27	92	3.31	12.03	59	8.72
28	96	4.08	16.32	39	12.24
29	135	6.33	3.31	7	died
30	102.5	4.50	15.09	45	10.59
31	72	7.14	13.05	108	5.91
17 completing		89.54	243.52	1020	153.98

Group I Lot 2 Iron(.5mg.)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. gained per 100 cc.
1	94	5.50	5.50	5	died
2	92	4.50	15.51	51	11.01
3	106	5.50	15.09	46	9.59
4	108	5.70	15.09	84	9.39
5	77	4.29	4.89	21	transferred
3 completing		15.70	45.69	181	29.99

Group I Lot 3 Copper(.05mg.) and Iron(.5mg)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. gained per 100 cc.
1	101.5	5.10	14.28	25	9.18
2	121.5	6.75	13.26	10	6.51

TABLE I. (continued)

Group I Lot 3 (continued) Copper(.05mg) and Iron(.5mg)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. gained per 100 cc.
3	112.5	5.70	13.26	10	7.56
4	102.5	4.50	13.26	18	8.76
4		22.05	54.06	63	32.01

Group I Lot 4 Manganese(.1mg), Copper(.05mg) Iron(.5mg)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. gained per 100 cc.
1	75	3.31	13.26	25	9.85

The conclusion drawn from these lots of Group I are shown in the summary given in the following form:

Lot	Supplement in mgs.	Number of rats	Days on Experiment (average)	Hemoglobin grams gain (average)	Days to gain 1 gm. Hemoglobin
1	Mn .1 Fe .5	17	60.0	9.06	6.62
2	Fe .5	3	60.3	9.99	6.03
3	Cu .05 Fe .5	4	15.7	8.00	1.96
4	Cu .05 Fe .5 Mn .1	1	25.0	9.85	2.54

The results showed that iron alone caused building of hemoglobin and manganese was inert. Since this was not in accord with the results obtained by Titus of this station in a previous experiment and no cause could be found for this difference it was decided to repeat the experiment. In order to be sure no copper could be present, the iron solution was retreated with H_2S gas under pressure for 24 hours, the excess H_2S removed by boiling and any CuS present was taken out by filtering. The filtrate and washings were diluted to the original volume.

The results of this second group are shown in the following Table II:

TABLE II.

Group II Lot 1. Manganese(.1mg) and Iron(.5mg)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. Gained per 100 cc.
1	130	5.50	5.10	67	-.40
2	114.5	3.66	7.95	67	4.29
3.	125	6.12	13.66	67	7.54
4	148.5	5.31	6.93	67	1.62
5	56	3.87	10.20	67	6.33
6	67	4.89	13.86	67	8.97
7	137.5	5.70	12.43	67	6.73
8	74	4.08	9.58	60	died
9	59	5.31	2.85	67	lost blood
10	82	5.39	4.89	67	-.50
11	123	5.70	8.16	51	2.46
12	69.5	5.10	8.97	51	3.87
13	80.5	5.10	8.58	51	3.48
14	136.5	5.91	9.99	51	4.08

TABLE II. (continued)

Group II Lot 1. (continued) Manganese(.1mg) and Iron(.5mg)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. gained per 100 cc.
15	103	5.70	13.05	51	7.35
16	64	3.31	8.47	51	5.16
17	86	4.50	5.91	51	1.41
18	86	4.50	7.14	51	2.64
19	58.6	4.08	14.88	51	10.80
20	85.5	4.08	8.47	51	4.39
18 counted		88.42	168.64	1043	80.02

Group II Lot 2 Iron (.5mg)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. gained per 100 cc.
1	91	5.91	6.33	55	.42
2	99	5.91	11.22	55	5.31
3	118	4.84	4.84	5	killed
4	80.5	5.10	8.58	55	3.48
5	75	6.91	7.14	49	died
6	79.5	4.29	8.58	55	4.29
7	86	4.29	8.97	39	4.68
8	85	4.89	9.58	39	4.69
9	87.5	4.50	9.78	39	5.28
10	88	4.08	13.26	39	9.18
8 counted		38.37	76.30	425	37.93

Group II Lot 3 Copper(.05mg) and Iron(.5mg)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. gained per 100 cc.
1	58	4.69	13.88	16	8.76
2	74	5.50	14.88	16	9.19
2 counted		10.19	28.76	32	17.95

TABLE II. (continued)

Group II Lot 4. Manganese(.1mg), Copper(.05mg), Iron(.5gm)

Number of rat	Weight Initial gms.	Hemoglobin Initial gms per 100 cc.	Hemoglobin Final gms. per 100 cc	Days on Expt	Hemoglobin gms. gained per 100 cc.
1	78	4.69	13.86	7	9.17
2	74	4.29	14.07	16	9.78
2 counted		8.98	27.93	23	18.95

The conclusion drawn from this second group is best summarized in the following tabular form:

Lot	Supplement in mgs.	Number of rats	Days on Experiment (average)	Hemoglobin grams gain (average)	Days to gain 1 gm. Hemoglobin
1	Mn .1 Fe .5	18	57.9	4.44	13.04
2	Fe .5	8	53.1	4.74	11.22
3	Cu .05 Fe .5	2	16.0	9.28	1.72
4	Cu .05 Fe .5 Mn .1	2	11.5	9.47	1.21

(1) This table shows that iron alone caused building of hemoglobin. This does not agree with the results obtained by Hart (5) or by Titus and co-workers (8), but does agree with the results obtained by Meyers (2), Mitchell (6) and Nelson.

(2) The rats receiving manganese in addition to iron did not rebuild their hemoglobin any faster than the group receiving iron alone, which indicates that the added manganese had no effect on hemoglobin building. This agrees with Hart (1), (5) but does not agree with the results of Titus (8).

(3) The rats receiving copper in addition to iron rebuilt their hemoglobin very rapidly. This beneficial action of copper is in accord with the results of all reported experiments.

CONCLUSIONS

There must be some factors which influence the building of hemoglobin which are not being controlled in this experiment. More work will have to be done to discover these unknown facts.

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